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STUDY OF PHENYLBUTAZONE TOXICITY IN AVIAN SPECIES

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ABSTRACT

A vulture crisis is an important environmental problem, which happened because of utilization of unsafe anti-inflammatory and analgesic drugs. The clinical profile of phenylbutazone is not very much different from other NSAID's. Despite of sharing pharmacological usefulness, phenylbutazone also shares unwanted effects which may eventually lead to the serious therapeutic complications and ecological imbalance. Therefore; we have aimed this study to evaluate the effects of toxic doses of phenylbutazone in broiler chickens. Two hundred and twenty five (225) healthy broiler chickens were reared up to 28 days and were divided into 5 groups each comprising 25 birds. On day 29 four groups were dosed 50mg/kg body weight twice a day intra-muscularly for 4 days. Food and water were provided *ad libitum*. A physical examination, toxicity and mortality rate were recorded daily. Blood samples were drawn to determine the serum values of aspartame transaminase (AST), alanine transaminase (ALT), uric Acid, alkaline phosphatase (ALP), and creatinine. Postmortem was performed on day 41. In second experiment other 100 birds were divided into 5 groups, each comprising 20 birds. One of the groups was injected I/M phenylbuazone 100 mg/kg twice a day. Postmortem was performed after medication on day 5. Based on the necropsy findings and biochemical analysis, phenylbuazone was not found to be safe in the avian species. Thus, it is suggested that the veterinary use of phenylbuazone should be avoided.

Keywords: Phenylbutazone toxicity, Broiler birds, LFTs

INTRODUCTION

Phenylbutazone; (3, 5-Pyrazolidinedione, 4-butyl-1, 2-diphenyl-Butazolidin, $C_{19}H_{20}N_2O_2$) is a white to off-white, odorless, crystalline powder. Soluble in alcohol, water, acetone and ether. It has similar anti-inflammatory effects and different toxicity profile as compared to the other salicylates.[1] Like aminopyrine, phenylbutazone can cause retention of sodium and chloride ion, edema, nausea, vomiting epigastric discomfort.[2],skin rashes, peptic ulcer hemorrhage[3] perforation, hypersensitivty reaction, serum sickness, ulcerative stomatitis, hepatitis, nephritis, aplastic anemia, leukopenia, agranulocytosis and thrombocytopenia[4] A number of deaths have also been reported, especially from aplastic anemia and agranulocytosis. Keeping the above in view, we aimed this study to investigate the toxicity and evaluate the safety of phenylbutazone in avian species to avoid hazards in wild life.

MATERIALS AND METHODS

The experiment was conducted at experimental sheds of the Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore. One hundred and fifty (150) day old broiler chicks collected from the "Pakistan Hatchery, Lahore" were

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vaccinated according to the vaccination schedule given in Table I. The phenylbutazone (Orient labs. Pvt. limited) was injected twice a day.

Table I: Vaccination schedule

Age	Vaccine	Route
6th day	Newcastle disease	Eye
15th day	Gumboro (I.B.D)	Oral*
21st day	Newcastle disease	Oral*
25th day	Gumboro (I.B.D)	Oral *

*With drinking water

MATERIALS AND METHODS

Experimental Design

On day 28, 75 birds were randomly divded into 2 groups comprising; group A with 50 and group B with 25 birds. On day 29, phenylbutazone I/M 50mg/kg body weight was injected twice a day up to four days indivdually to each bird of group A, for four consecutive days. Group B was kept as control. The remaining 75 birds were divided into two groups C with 50 and D with 25 birds. Each bird of group C was injected phenylbuazone I/M 100 mg/kg body weight twice a day for four days and group D was kept as controlled without medication. Food and water was provided *ad libitum*. A daily basis record of physical examination, sign and symptoms and toxicity was maintained regularly.

Sample Schedule and Parameters Determined

The sampling schedule and different parameters were investigated by Asif et al[5]. Three ml blood sample from birds of each group (A, B, C and D) was collected before the start of medication on day 29. Then blood sample from the same birds were drawn from wing vein (vena cutanea ulnaris) on days 33, 37 and 41 after medication for determination of serum values of following parameters; aspartate transaminase, alanine transaminase,[6] uric acid, alkaline phosphatase (ALP)[7] concentration of creatinine in serum[8].

Clinical Findings and Statistical Analysis

The clinical findings mortality and postmortem were recorded during the study. The collected data were analyzed statistically with one way analysis of variance.[9] On days 41 and 47 the postmortem were done. Three parameters; postmortem, liver, kidney biopsies and staining of specimens were examined.

RESULTS

The biochemical parameters including uric acid, creatinine, alanine transaminase, aspartate transaminase and alkaline phosphatase were noted for test and controlled birds before and after phenylbutazone dose. The necropsy findings of experimental chicks were also recorded, as given below.

Biochemical Parameters of Phenylbutazone

Phenylbutazone I/M 50mg/kg and 100 mg/kg body weight were injected to each bird of group A and B respectively; twice a day for four days and following parameters were measured.

Uric Acid: As shown in Table II, the mean values of uric acid of phenylbutazone were 5.960040 mg/dl, 5.130200 mg/dl, 5.532480 mg/dl and 5.234160 mg/dl before medication, 1st, 5th and 9th days after medication, respectively. There was no significant difference in the mean values of uric acid of phenylbutazone Table III.

Table II. Biochemical parameters of phenylbutazone group. Mean±SEM, N=5

Time of sample collection	Uric Acid mg/dl	Creatinine mg/dl	ALT µg/L	AST µg/L	ALP µg/L
Before medication	5.960040 ± .331098	1.334880±.109400	10.979760 ± .434199	184.13982 ± 6.051957	29.252640 ±2.707351
1st day after medication	5.130200 ± .218903	1.283580 ±.130102	15.639820 ± 1.314115	299.63830 ± 4.953551	59.784000 ±2.074060
5th day after medication	5.532480 ± .292441	1.269640 ±.113400	15.633520 ± 1.985212	242.31240 ± 7.902804	65.208000 ±2.053547
9th day after medication	5.234160 ± .363122	1.151620±.105056	11.045560 ± .583005	187.74400 ±14.487782	59.564000 ±4.113037

Table III. Statistical Analysis of Phenylbutazone Data

Parameters		Sum of Squares	Df	Mean Square	F	Sig.
Uric acid	Between groups	2.075	3	.692	1.476	.259
	Within groups	7.498	16	.469		
	Total	9.573	19			
Creatinine	Between groups	9.001E-02	3	3.000E-02	.455	.718
	Within groups	1.056	16	6.599E-02		
	Total	1.146	19			
ALT	Between groups	106.918	3	35.639	4.601	.017
	Within groups	123.928	16	7.745		
	Total	230.846	19			
AST	Between groups	44401.552	3	14800.517	35.502	.000
	Within groups	6670.280	16	416.892		
	Total	51071.832	19			
ALP	Between groups	4006.317	3	1335.439	32.606	.000
	Within groups	655.312	16	40.957		
	Total	4661.629	19			

Biochemical Parameters in Control Group

No medication was given to group B and D.

Uric Acid: As shown in Table II, the mean values of uric acid of normal or control bird were 5.031800 mg/dl, 4.776720 mg/dl, 5.479140 mg/dl and 4.874880 mg/dl at 1st, 5th and 9th day, respectively. There was no significant difference in the mean values of uric acid of control birds.

Creatinine: The mean values of creatinine in control birds were 1.052080 mg/dl, 0.972660 mg/dl, 1.134440 mg/dl and 1.066040 mg/dl at 1st, 5th and 9th day, respectively. There was no significant difference in the mean values of creatinine in control birds.

Alanine Transaminase (ALT): The mean values of alanine transaminase of control birds were found to be 10.149740 µ/L, 10.205000 µ/L, 10.269660 µ/L and 10.351780 µ/L at 1st, 5th and 9th day, respectively. There was no significant difference in the mean values of ALT in controlled birds.

Aspartate Transaminase (AST): As shown in table IV, the mean values of aspartate transaminase of control birds were found to be 193.555 µ/L, 199.435 µ/L, 158.98660 µ/L and 166.81000 µ/L. There was no significant difference in the mean values of aspartate transaminase of control birds.

Alkaline Phosphatase (ALP): The mean values of alkaline phosphatase of piroxicam were 27.252000 µ/L, 34.470000 µ/L, 33.548000 µ/L and 27.824400 µ/L. There was no significant difference in the mean values of alkaline phosphatase of control birds.

Necropsy of Experimental Chicks in Different Groups

The birds were slaughtered at the end of experiment and different lesions in kidney, liver and muscles were recorded. Each bird of group C was injected with I/M phenylbutazone 100 mg/kg body weight, twice a day for four days. Each bird of group C was injected with I/M phenylbutazone 100 mg/kg body weight, twice a day for four days. Findings are given in Table IV.

Table IV: Necropsy of various drugs in broilers chicks, N=15

Drug	Dose	Postmortem lesions		
		Site of injection	Liver	Kidney
Phenylbutazone	50 mg/kg	10/15	7/15	0/15
	100 mg/kg	15/15	12/15	0/15
Control	No medication	0/15	0/15	0/15
	No medication	0/15	0/15	0/15

Control Group: Group B and D were kept control without medication and the findings are given in Table IV.

DISCUSSION

Phenylbutazone is a widely used non-steroidal anti-inflammatory drug with little anti-pyretic and analgesic effects. The adverse effects associated with Phenylbutazone include; peptic ulcer hypersensitivity reaction ulcerative stomatitis, hepatitis nephritis aplastic anemia and agranulocytosis. The current study showed that there was no toxic effect on kidneys in broiler chicks as indicated by the necropsy finding and biochemical analysis of serum uric acid and creatinine. GIT toxicity, edema of small intestine, erosions, and ulcers of large colon and development of renal crest necrosis were observed.[4] The results of the present study differed from the above observations which might be due to different species used for experiment. The findings of present study are partially in agreement with the observations of Kari and co-workers,[3] who investigated the toxic effects of phenylbutazone on the kidneys and liver in mice and reported different lesions as hemorrhage, centrilobular cytomegaly, fatty metamorphosis, cellular degeneration, coagulative necrosis and clear cell foci in liver. They also observed lesions in kidney which were not found in the present study which might be due to different animal species used for experiments.

Hepatotoxicity was observed in phenyl butazone treated group, while there no nephrotoxicity was found. Muscle necrosis was observed in almost all of the birds at the site of injection. There was no significant difference in serum uric acid and creatinine levels which indicated that phenylbutazone had no toxic effects on kidneys, but there was significant difference in the values of serum ALT, AST and ALP in phenylbutazone treated group as stated by Embert, 1986[10]

The levels of ALT, AST and ALP might be elevated due to cellular degeneration or destruction in liver muscles and acute hepato cellular necrosis or bilary obstruction. Similar observations were recorded in the present study. It could be concluded that phenyl butazone is hepato toxic in avan species even at the dosage rate of 50 mg/kg body weight.

The postmortem of the control group revealed no abnormalities particularly in liver, kidneys and muscles. Similarly there was no significant difference in the serum values of uric acid and creatinine ALT, AST and ALP in the samples collected at the different times during experiments in the group.

CONCLUSION

The phenylbutazone was studied for its toxicity in broiler chicks. No mortality was recorded in all groups. Based on the necropsy findings and biochemical analysis it was found that phenylbutazone was not safe drug in the avan species. Keeping in view the environmental problem (vulture's crises) it could be recommended that phenylbutazone having good pharmacological effects in human should be avoided in veterinary practice.

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